Isoflurane activates intestinal sphingosine kinase to protect against bilateral nephrectomy-induced liver and intestine dysfunction

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Kim M, Park SW, Kim M, D’Agati VD, Lee HT. Isoflurane activates intestinal sphingosine kinase to protect against bilateral nephrectomy-induced liver and intestine dysfunction. Am J Physiol Renal Physiol 300: F167–F176, 2011. First published October 20, 2010; doi:10.1152/ajprenal.00467.2010.—Acute kidney injury (AKI) frequently leads to systemic inflammation and extrarenal dysfunction. Volatile anesthetics are potent anti-inflammatory agents and protect against renal ischemia-reperfusion injury. Here, we sought to determine whether isoflurane, a commonly used volatile anesthetic, protects against AKI-induced liver and intestinal injury, the mechanisms involved in this protection, and whether this protection was independent of the degree of renal injury. Bilateral nephrectomy-induced AKI under pentobarbital sodium anesthesia led to severe hepatic and intestinal injury with periporal hepatocyte vacuolization, small intestinal necrosis, apoptosis, and proinflammatory mRNA upregulation. In contrast, isoflurane anesthesia reduced hepatic and intestinal injury after bilateral nephrectomy. Mechanistically, isoflurane anesthesia upregulated and induced small intestinal crypt sphingosine kinase-1 (SK1) as SK1 mRNA, protein, and enzyme activity increased with isoflurane treatment. Furthermore, isoflurane failed to protect mice treated with a selective SK inhibitor (SKI-II) or mice deficient in the SK1 enzyme against hepatic and intestinal dysfunction after bilateral nephrectomy, demonstrating the key role of SK1. Therefore, in addition to its potent anesthetic properties, isoflurane protects against AKI-induced liver and intestine injury via activation of small intestinal SK1 independently of the effects on the kidney. These findings may help to elucidate the cellular signaling pathways underlying volatile anesthetic-mediated hepatic and intestinal protection and result in novel clinical applications of volatile anesthetics to attenuate perioperative complications arising from AKI.

Acute kidney injury; acute renal failure; apoptosis; inflammation; necrosis; sphingosine-1-phosphate

Acute kidney injury (AKI) is a major clinical problem during the perioperative period (24) and often leads to multiorgan dysfunction and systemic inflammation, contributing to significant morbidity and mortality in hospitalized patients. Hepatic dysfunction occurs frequently in patients with AKI and leads to other complications such as intestinal barrier dysfunction, respiratory failure, and multiorgan failure frequently complicated by sepsis (10). Recently, we showed that AKI leads to rapid hepatic and intestinal injury in mice with increased inflammation, apoptosis, and necrosis mediated by upregulation of the proinflammatory cytokines TNF-α, IL-17A, and IL-6 (46).

Virtually all patients undergoing general anesthesia in the United States are exposed to volatile anesthetics, and therefore volatile anesthetics are one of the most widely used drugs during the perioperative period. Fortuitously, in addition to their analgesic and anesthetic properties, volatile anesthetics produce tissue protection in several organs including the heart (21), kidney (33), liver (50), and brain (59). We previously demonstrated that volatile anesthetics protected against renal ischemia-reperfusion (IR) injury in vivo by reducing renal cell necrosis and attenuating inflammatory leukocyte and cytokine attack (32). However, the detailed mechanisms and exact cellular targets of volatile anesthetics to mediate this multiorgan protection remain to be fully elucidated. Most volatile anesthetics are lipophilic molecules (3) and are known to modulate the plasma membrane and sphingomyelin metabolism in the renal cortex (37). One of the major components of sphingomyelin metabolism involves sphingosine kinase (SK)-mediated production of sphingosine-1-phosphate (S1P). Modulation of SK and S1P signaling is particularly attractive as this pathway has been shown to be an important regulator in promoting cell growth and survival and the inhibition of apoptosis (17, 53).

In this study, we aimed to 1) determine whether volatile anesthetics protect against liver and intestine injury after AKI; 2) demonstrate whether this protection was independent of the effects of volatile anesthetics on the kidney; and if so 3) identify the cell type targeted by volatile anesthetics to mediate multiorgan protection; and 4) test whether volatile anesthetics activate SK/S1P signaling. Therefore, we used a murine model of bilateral nephrectomy to test the hypothesis that volatile anesthetics activate the SK1/S1P pathway in the small intestine to protect against AKI-induced hepatic and intestinal injury. We demonstrate here that isoflurane reduced hepatic and intestinal proinflammatory cytokine upregulation and intestinal apoptosis and necrosis via induction of SK1 in small intestinal crypts.

Materials and Methods

Materials. Isoflurane [2-chloro-2-(difluoromethoxy)-1,1,1-trifluoro-ethane] was purchased from Abbott Laboratories (North Chicago, IL). SKI-II [4-[[4-(4-chlorophenyl)-2-thiazolyl]amino]phenol] was purchased from Tocris Bioscience (Ellisville, MO). Unless otherwise specified, all other reagents were purchased from Sigma (St. Louis, MO).

Murine model of bilateral nephrectomy. All animal protocols were approved by the Institutional Animal Care and Use Committee of Columbia University (New York, NY). We used male C57BL/6 (20–25 g, Harlan, Indianapolis, IN) or SKIKO (20 to 25 g, kindly provided by R. L. Proia, NIH, Bethesda, MD) mice. The generation and initial characterization of SK1 knockout (SK1KO) mice have been described previously (2). These mice are congenically derived on a C57BL/6 background.

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In our model of bilateral nephrectomy, mice were initially anesthetized with intraperitoneal pentobarbital sodium (50 mg/kg body wt, or to effect, Henry Schein Veterinary, Indianapolis, IN), and after midline laparotomy, both kidneys were removed. After closure of the abdomen in two layers, the mice were then exposed to an additional 4 h of equiptent doses of either pentobarbital sodium or isoflurane [1.2% or 1 minimum alveolar concentration (MAC; defined as the concentration of volatile anesthetic in the lungs that is needed to prevent movement in 50% of subjects in response to a painful stimulus)] as described previously (33). The mice were placed on a heating pad under a warming light to maintain body temperature ~37°C.

To test the effects of SK inhibition, SKI-II (50 mg/kg) was administered to mice subcutaneously 15 min pre- and 4 h postnephrectomy. SK-I-I is an SK-selective inhibitor with minimal effects on other kinases (11), and this dose was shown to have effective inhibition of activity without significant toxicity (12).

For experiments involving sham operation or bilateral nephrectomy, all samples (including plasma and tissue) were collected from mice 5 h after sham operation or bilateral nephrectomy, as previous studies demonstrated that liver and intestinal injury peaked at this time point after bilateral nephrectomy (46). For experiments involving sham exposure to pentobarbital sodium or isoflurane (SK mRNA and protein expression, SK1 immunohistochemistry, SK activity, and S1P measurement), mice were exposed to 4 h of pentobarbital sodium or isoflurane (without sham operation or bilateral nephrectomy) and allowed to recover for 1 h. Samples were collected 5 h after initial anesthetic exposure.

**Plasma alanine aminotransferase activity and creatinine level.**

Plasma alanine aminotransferase (ALT) activity was measured using an Infinity ALT assay kit according to the manufacturer’s instructions (Thermo Fisher Scientific, Waltham, MA). Plasma creatinine was measured by an enzymatic creatinine reagent kit according to the instructions provided by the manufacturer. 

Histological analysis of liver and small intestine injury. For histological preparations, liver or small intestine (jejunum and ileum) tissues collected from mice were washed in ice-cold PBS and fixed overnight in 10% formalin. After automated dehydration through a graded alcohol series, tissues were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin-eosin (H&E).

**Detection of small intestinal apoptosis after bilateral nephrectomy.**

We detected small intestinal apoptosis with terminal deoxynucleotidyl transferase biotin-dUTP nick end-labeling (TUNEL) staining. In situ labeling of fragmented DNA was performed with TUNEL stain (green fluorescence) using a commercially available in situ cell death detection kit (Roche, Indianapolis, IN) according to the instructions provided by the manufacturer.

![Fig. 1. Plasma alanine aminotransferase (ALT; U/l) after bilateral nephrectomy.](http://ajprenal.physiology.org/)

**Table 1. Primers used to amplify mRNAs encoding SK1, SK2, GAPDH, and proinflammatory cytokines based on published GenBank sequences for mice**

<table>
<thead>
<tr>
<th>Primers</th>
<th>Accession No.</th>
<th>Sequence (Sense/Antisense) Product Size, bp Cycle No. Annealing Temperature, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse SK1</td>
<td>NM_011451 (variant 1)</td>
<td>5'-GATCATGATGAATTCCAAGAAC-3' 337 22 64</td>
</tr>
<tr>
<td>Mouse SK2</td>
<td>NM_025367 (variant 2)</td>
<td>5'-ACATGCTGGCTTGGTGGTGGTG-3' 437 23 68</td>
</tr>
<tr>
<td>Mouse MIP-2</td>
<td>X53798</td>
<td>5'-CAGGAGGACACTAGGTAGTC-3' 282 28 60</td>
</tr>
<tr>
<td>Mouse ICAM-1</td>
<td>X52264</td>
<td>5'-CTGGGTTGACGTCGTCG-3' 409 21 60</td>
</tr>
<tr>
<td>Mouse TNF-α</td>
<td>X02611</td>
<td>5'-ACTGCTGGCTACCTCATCC-3' 290 24 65</td>
</tr>
<tr>
<td>Mouse MCP-1</td>
<td>NM_011333</td>
<td>5'-ACCTGCTGGCTACCTCATCC-3' 312 22 60</td>
</tr>
<tr>
<td>Mouse IL-6</td>
<td>NM_031168</td>
<td>5'-CCGAGAAGAGAGACTTCAG-3' 431 20 62</td>
</tr>
<tr>
<td>Mouse IL-17</td>
<td>NM_010552</td>
<td>5'-GCCAGAAGAGAGACTTCAG-3' 248 32 66</td>
</tr>
<tr>
<td>Mouse GAPDH</td>
<td>M32599</td>
<td>5'-ACCACACTCGATGCCATCCAC-3' 450 15 65</td>
</tr>
</tbody>
</table>

SK, sphingosine kinase; MCP-1, monocyte chemoattractant protein 1; MIP-2, macrophage inflammatory protein 2. Respective anticipated RT-PCR product size, PCR cycle number for linear amplification, and annealing temperatures used for each primer are also provided.
Assessment of liver and small intestine inflammation and SK expression. Liver and intestine inflammation was determined by measuring mRNA encoding markers of inflammation, including IL-6, IL-17A, ICAM-1, monocyte chemoattractive protein 1 (MCP-1), macrophage inflammatory protein 2 (MIP-2), and TNF-α (Table 1). In addition, SK1 and SK2 mRNA levels were measured. Semiquantitative real-time RT-PCR was performed as described (34).

Fig. 2. Isoflurane protects against liver injury after bilateral nephrectomy. Representative photomicrographs are shown of the livers (from 4 experiments, hematoxylin and eosin staining, magnification of ×400) of mice subjected to sham operation (A) or to bilateral nephrectomy followed by exposure to 4 h of pentobarbital sodium (B and D) or 1.2% isoflurane (C). Tissues were collected 5 h after sham operation or bilateral nephrectomy. With pentobarbital sodium exposure after bilateral nephrectomy, the livers of mice demonstrate marked vacuolization injury (B; for periportal area magnified in D, arrows highlight vacuolization). Isoflurane exposure after bilateral nephrectomy mitigates these injuries (C).

Vascular permeability of liver and small intestine after bilateral nephrectomy. Changes in liver and small intestine vascular permeability were assessed by quantitating extravasation of Evans blue dye (EBD) into the tissue as described previously (48).

Immunoblotting analyses of small intestine. Small intestinal tissues in mice were collected and homogenized in lysis buffer [20 mM HEPES (pH 7.4), 2 mM EGTA, 1 mM DTT, 1% Triton X-100, 10%

Fig. 3. Isoflurane protects against small intestinal injury after bilateral nephrectomy. Representative photomicrographs are shown of the small intestines (from 4 experiments, hematoxylin and eosin staining, magnifications of ×200 and ×600) of mice subjected to bilateral nephrectomy followed by exposure to 4 h of pentobarbital sodium (A and B) or 1.2% isoflurane (C and D). Tissues were collected 5 h after bilateral nephrectomy. With pentobarbital sodium exposure after bilateral nephrectomy, the intestines of mice demonstrate marked epithelial necrosis (A and B, arrows), villous swelling (B), and numerous apoptotic bodies (B, inset, arrowheads). Isoflurane exposure after bilateral nephrectomy mitigates these changes (C and D).
glycerol, and protease inhibitor cocktail (Calbiochem, La Jolla, CA) on ice with a glass homogenizer. The homogenates were centrifuged for 20 min at 16,000 g. The supernatant was collected and used for immunoblotting as described earlier (25). The samples (50–100 µg protein/lane) were separated on 9 or 12% SDS-PAGE and then transferred to Immobilon membranes (Millipore, Bedford, MA). The membranes were blocked with blocking buffer (5% nonfat dry milk in TBS containing 0.1% Tween 20) and incubated overnight with polyclonal anti-SK1 (3297; 1:1,000 dilution; Cell Signaling, Beverly, MA), anti-SK2 (ab37977; 1:2,000 dilution; Abcam, Cambridge, MA), or monoclonal anti-β-actin (A5316; 1:5,000 dilution) antibodies diluted in blocking buffer at 4°C. After being washed, the membranes were incubated with horseradish peroxidase-conjugated donkey anti-rabbit or sheep anti-mouse (1:5,000 dilution; ECM Bioscience, Versailles, KY) antibodies for 1 h at room temperature. Finally, the membranes were detected with enhanced chemiluminescence immunoblotting detection reagents (Amersham, Piscataway, NJ) with subsequent exposure to a CCD camera coupled to a UVP Bio-imaging System (Upland, CA). The band intensities of the immunoblots were within the linear range of exposure for all experiments.

Immunohistochemical detection of SK1. We used immunohistochemistry to detect small intestinal SK1. Paraffin-embedded intestine sections from mice were deparaffinized in xylene and rehydrated through a graded ethanol series to water. Antigen retrieval was performed in 95°C 10 mM sodium citrate (pH 6.0) for 10 min. Endogenous peroxidase activity for all sections was quenched with 0.3% H2O2 while nonspecific binding was reduced by blocking with buffer containing 1% bovine serum albumin. The slides were stained for SK1 in sequential incubations with rabbit anti-mouse SK1 primary antibody (AP7237, 1:50 dilution; Abgent, San Diego, CA) overnight at 4°C, horseradish peroxidase-conjugated goat anti-rabbit IgG (PI-1000, 1:200 dilution, Vector Laboratories, Burlingame, CA) for 1 h at room temperature, and diaminobenzidine reagent (Vector Laboratories) for 2 min. A rabbit IgG (I-1000, Vector Laboratories) was used at the same concentration as the primary antibody as a negative isotype control for all experiments.

HPLC detection of S1P. S1P levels were measured in the small intestines of mice using HPLC as described (29).

Protein determination. Protein content was determined with the Thermo Scientific bicinchoninic acid protein assay reagent with bovine serum albumin as a standard.

Statistical analysis. The data were analyzed with Student’s t-test when means between two groups were compared. One-way ANOVA plus Tukey’s post hoc multiple comparison test was used when multiple groups were compared. In all cases, a probability statistic <0.05 was taken to indicate significance. All data are expressed throughout the text as means ± SE.

RESULTS

Isoflurane protects against acute hepatic injury after bilateral nephrectomy in mice. All animals awakened from anesthesia following bilateral nephrectomy with pentobarbital sodium or isoflurane exposure. There were no observable differences in the condition and health of the mice between the two groups. As demonstrated previously (30), there were no changes in systemic arterial blood pressure, renal blood flow, or core body temperature between pentobarbital sodium- and

Fig. 4. Isoflurane protects against intestinal apoptosis after bilateral nephrectomy. Representative fluorescence photomicrographs (of 4 experiments) are shown illustrating apoptotic nuclei [terminal deoxynucleotidyl transferase biotin-dUTP nick end-labeling (TUNEL) fluorescence staining, green] in the small intestine. The left side of each panel depicts a ×100 fluorescence photomicrograph with a highlighted area (white box) enlarged to ×400 on the right side of the panel. Mice were exposed to 4 h of pentobarbital sodium after sham operation (A) or bilateral nephrectomy (C) or to 4 h of 1.2% isoflurane after sham operation (B) or bilateral nephrectomy (D). Tissues were collected 5 h after sham operation or bilateral nephrectomy.

5 hrs after Sham Surgery or Bilateral Nephrectomy

100X

200X

Pentobarbital

Isoflurane

Sham

Bilateral Nephrectomy

A

B

C

D

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Isoflurane-anesthetized mice. Five hours after bilateral nephrectomy, there was a rise in plasma creatinine (mg/dl) with pentobarbital sodium exposure (Cr = 1.23 ± 0.08, n = 10, P < 0.001) compared with sham-operated mice (Cr = 0.45 ± 0.03, n = 6). With a complete lack of renal function, however, there was no significant reduction in plasma creatinine with isoflurane exposure (Cr = 1.12 ± 0.04, n = 8, not significant).

Mice developed acute hepatic injury after bilateral nephrectomy with pentobarbital sodium exposure as indicated by a rise in plasma ALT above sham levels (Fig. 1A). In contrast, isoflurane exposure after bilateral nephrectomy protected against liver injury with a significant reduction in plasma ALT levels (Fig. 1A).

To evaluate the role of SK in mediating the protective effects of isoflurane, we treated some animals with a selective SK inhibitor SKI-II before bilateral nephrectomy. SKI-II administration had no detrimental effects on renal function (Cr = 0.43 ± 0.04, n = 3) in sham-operated mice. Plasma ALT levels increased in SKI-II-treated mice exposed to pentobarbital sodium after bilateral nephrectomy compared with sham-operated mice (Fig. 1B). There was no reduction in plasma ALT levels with isoflurane exposure. In addition, we utilized a strain of mice deficient in SK1 enzyme. SK1KO mice exposed to pentobarbital sodium after bilateral nephrectomy had elevated plasma ALT levels compared with sham-operated mice with no reduction in plasma ALT levels with isoflurane exposure (Fig. 1B). Taken together, these data indicate that the protective effects of isoflurane on hepatic function after bilateral nephrectomy are abolished with inhibition of SK1 enzyme in mice.

Isoflurane exposure reduces hepatic vacuolization and small intestinal necrosis and apoptosis. In Figs. 2 and 3, the protective effects of isoflurane anesthesia are further supported by representative histological slides collected from mice after sham operation or bilateral nephrectomy. Pentobarbital sodium exposure after bilateral nephrectomy led to nuclear and cytoplasmic degenerative changes, cellular vacuolation, leukocyte infiltration, and congestion in the liver (Fig. 2, B and D) and profound epithelial necrosis, villous swelling, and apoptosis in the small intestine (Fig. 3, A and B). Isoflurane exposure after bilateral nephrectomy significantly attenuated these injuries in the liver (Fig. 2C) and small intestine (Fig. 3, C and D).

We failed to detect significant TUNEL-positive cells in small intestinal sections from sham-operated mice exposed to pentobarbital sodium (Fig. 4A) or isoflurane (Fig. 4B). Mice exposed to pentobarbital sodium after bilateral nephrectomy (Fig. 4C) showed many TUNEL-positive cells in the small intestine (representative of 4 experiments) whereas mice exposed to isoflurane after bilateral nephrectomy (Fig. 4D) had a reduction in TUNEL-positive cells in the small intestine.

### Fig. 5. Isoflurane Protects Against Bilateral Nephrectomy-Mediated Hepatic and Intestinal Proinflammatory mRNA Upregulation in C57BL/6 Mice, but Not in Mice Lacking SK-1.

C57BL/6 mice (A) or SK1KO (B) mice were subjected to bilateral nephrectomy followed by exposure to 4 h of pentobarbital sodium (open bars) or 1.2% isoflurane (filled bars). Liver and small intestine tissues were collected 5 h after bilateral nephrectomy. Densitometric quantifications of band intensities relative to GAPDH from RT-PCR reactions; n = 4/group. *P < 0.05 vs. appropriate pentobarbital sodium group.
C57BL/6, but not SK1KO, mice exposed to isoflurane after bilateral nephrectomy show reduced proinflammatory gene expression in the liver and small intestine. We measured proinflammatory mRNA expression in the livers and intestines of C57BL/6 mice after sham operation or bilateral nephrectomy. We found increased mRNA expression of TNF-α, ICAM-1, IL-17A, MCP-1, MIP-2, and IL-6 in the livers and small intestines of mice exposed to pentobarbital sodium after bilateral nephrectomy compared with sham-operated mice (46). When mice were exposed to isoflurane after bilateral nephrectomy, there was significantly reduced expression of some proinflammatory mRNAs (TNF-α, IL-17A, and MIP-2) in both the liver and intestine compared with pentobarbital sodium exposure (Fig. 5A). In contrast, MCP-1 and ICAM-1 expression were not reduced with isoflurane exposure after bilateral nephrectomy in both the liver and intestine. Interestingly, isoflurane exposure decreased IL-6 expression in the liver, but not intestine, after bilateral nephrectomy. We also measured proinflammatory mRNA expression in the livers and intestines of SK1KO mice after sham operation or bilateral nephrectomy. In contrast to C57BL/6 mice, there were no reductions in proinflammatory mRNAs in the livers or intestines of SK1KO mice exposed to isoflurane after bilateral nephrectomy compared with pentobarbital sodium-exposed mice.

Isoflurane exposure decreases hepatic and small intestinal vascular permeability after bilateral nephrectomy. We measured liver and small intestinal vascular permeability after sham operation or bilateral nephrectomy using EBD. EBD binds to plasma proteins, and its appearance in extravascular tissues reflects an increase in vascular permeability (5). Bilateral nephrectomy caused significant increases in vascular permeability as measured by increased EBD content compared with sham-operated mice in the liver, jejunum, and ileum (Fig. 6). Vascular permeability was significantly decreased with isoflurane exposure after bilateral nephrectomy in the liver, jejunum, and ileum.

Isoflurane increases small intestinal SK1 mRNA and protein expression. We measured SK mRNA and protein expression in the small intestines of sham mice (without operation) after exposure to either pentobarbital sodium or isoflurane. Isoflurane exposure increased small intestinal SK1 mRNA expression (Fig. 7) and protein expression (Fig. 8) in mice compared with pentobarbital sodium exposure. However, there were no changes in SK2 mRNA or protein expression in the small intestine after isoflurane exposure (Figs. 7 and 8). In addition, the livers of sham mice exposed to pentobarbital sodium or isoflurane demonstrated a similar increase in SK1 mRNA and protein expression with no change in SK2 mRNA or protein (data not shown).

Isoflurane increases small intestinal crypt SK1 expression. We used immunohistochemistry to detect SK1 expression in sham mice. Isoflurane exposure increased staining for SK1 in the small intestine. Specifically, we saw increased expression of SK1 protein in the small intestinal crypts with enhanced staining in the nuclei and cytoplasm. (Fig. 9, C and D, representative of 4 experiments), compared with pentobarbital sodium exposure (Fig. 9, A and B). Staining specificity was confirmed with negative, isotype-specific control antibodies (data not shown).

Isoflurane increases small intestinal SK activity and S1P levels. We measured SK enzymatic activity and S1P levels in the small intestines of sham mice (without operation) after exposure to either pentobarbital sodium or isoflurane. The small intestines of mice exposed to isoflurane demonstrated higher SK activity compared with the small intestines of mice exposed to pentobarbital sodium (Fig. 10A). Correspondingly, small intestinal S1P levels were higher in mice after isoflurane exposure than after pentobarbital sodium exposure (Fig. 10B). However, we were unable to detect an increase in SK activity in the livers of sham mice exposed to pentobarbital sodium (relative SK activity = 1.00 ± 0.03, n = 4) compared with isoflurane (relative SK activity = 1.01 ± 0.08, n = 4).

**DISCUSSION**

In this study, we have demonstrated that a clinically relevant concentration of isoflurane (1.2% or 1 MAC) administered to
mice after bilateral nephrectomy reduced hepatic and small intestinal injury by reducing inflammation and apoptosis and improving vascular permeability. We show that isoflurane-mediated liver and intestine protection did not require intact renal function and was independent of the effects of volatile anesthetics on the kidney. In addition, isoflurane induced small intestinal SK1 mRNA, protein expression, and enzymatic activity with higher small intestinal S1P levels. We also confirmed the critical role of SK1 as isoflurane failed to protect mice treated with an SK inhibitor (SKI-II) or mice lacking the SK1 enzyme.

Perioperative AKI remains a clinically significant problem, and the development of postoperative AKI (1) and extrarenal dysfunction following AKI, including hepatic and respiratory failure, were independent predictors of in-hospital mortality (40). The inflammatory response following AKI has both local and systemic effects including endothelial and proximal tubule injury with release of proinflammatory cytokines and chemokines and the recruitment of leukocytes such as macrophages, neutrophils, and T lymphocytes (6). It is clear that the effects of AKI are not limited to the kidney but frequently spread to distant organs including the lung (31), heart (27), brain (36), and liver (15).

We have previously demonstrated the protective role of volatile anesthetics in renal IR injury (33). Here, we aimed to determine whether volatile anesthetics mediated protection in the liver and small intestine after AKI and whether this protection was independent of the protective effects of volatile anesthetics in the kidney. Therefore, we used a model of nonischemic AKI with bilateral nephrectomy in mice. Bilateral nephrectomy can be used to distinguish the effects of impaired renal function from those of renal IR injury (31). Both renal IR and bilateral nephrectomy lead to extrarenal dysfunction such as lung injury (28, 31) and hepatic injury (15). However, differences in the genomic response (18) and cytokine response (19) between renal IR and bilateral nephrectomy have been described. Serum levels of keratinocyte-derived chemokine and granulocyte-macrophage colony-stimulating factor increased significantly after renal IR but not bilateral nephrectomy (19).
Previous studies showed rapid hepatic injury and inflammation after ischemic or nonischemic AKI (15, 46). In addition, we demonstrated massive small intestinal injury (apoptosis, inflammation, and necrosis) after ischemic or nonischemic AKI (46). The gut has long been suspected to play a key role in initiating the early hyperdynamic response to sepsis (58), and recently we demonstrated that the inflammatory mediator IL-17A was released by small intestinal crypt Paneth cells after AKI in mice (47). IL-17A released from Paneth cells caused hepatic injury and a proinflammatory cascade, including upregulation of TNF-α and IL-6. In addition, hepatic and small intestinal inflammation was attenuated when mice were treated with antibodies to TNF-α, IL-17A, or IL-6 or in mice lacking TNF-α, IL-17A, or IL-6 (46). In this study, isoflurane decreased the mRNA expressions of proinflammatory cytokines MIP-2, IL-17A, and TNF-α in the liver and small intestine after bilateral nephrectomy.

Indeed, the anti-inflammatory effects of volatile anesthetics have been demonstrated as they decreased the inflammatory response of alveolar macrophages to endotoxin (54) and protected mice against zymosan-induced inflammation and acute lung injury (41). IL-17A, in particular, produced primarily by a subset of T cells known as Th17 cells, is involved in innate immune defense and inflammation (45). IL-17A-producing Th17 cells can be found in the intestinal lamina propria of healthy mice (20) but rarely in the spleen, mesenteric lymph nodes, or Peyer’s patches (4). In addition, Paneth cell-derived IL-17A mediated TNF-α-induced shock (56), and we showed that Paneth cell IL-17A production increases after AKI, leading to higher levels of additional cytokines, including TNF-α (47), which is a mediator of the inflammatory cascade seen after AKI (9). In this study, we demonstrate upregulation of intestinal crypt SK1 and increased S1P synthesis by isoflurane. Taken together, we propose that intestinal S1P generation with subsequent reductions in IL-17A and TNF-α, both produced in intestinal crypts, mediate the anti-inflammatory effects of volatile anesthetics, potentially leading to multiorgan protection.

The expression of IL-6 after bilateral nephrectomy was reduced with isoflurane exposure in the liver, but not in the small intestine, demonstrating a divergent effect of isoflurane on the liver and small intestine. IL-6 was involved in the progression of liver inflammation to hepatocellular carcinoma (42) but was shown to protect enterocytes against cell death and apoptosis and mice against intestinal IR injury (22). Therefore, selective reduction of hepatic IL-6 generation without concomitant reduction of intestinal IL-6 would explain potent hepatic and intestinal protection with isoflurane treatment. The expressions of MCP-1 and ICAM-1 did not change with isoflurane exposure after bilateral nephrectomy, perhaps reflecting the importance of intact renal function to better metabolize and excrete inflammatory mediators affecting the expression of these two cytokines, as studies in chronic renal failure patients suggest the importance of renal clearance of proinflammatory mediators (49). Moreover, we further demonstrate the importance of SK1 in mediating the anti-inflammatory effects of isoflurane as isoflurane exposure failed to reduce expression of proinflammatory mediators in both the liver and intestine in mice lacking SK1.

Volatile anesthetic-mediated protection has been demonstrated in organ systems such as the brain (59), heart (21), and lung (41), and the protective effects of volatile anesthetics in the kidney (30), HK-2 cells (29), and brain (59) may involve activation of the SK/S1P pathway. With this knowledge, we hypothesized that volatile anesthetic-mediated activation of SK1/S1P signaling in the small intestine would protect against extrarenal organ dysfunction following bilateral nephrectomy. Using both a pharmacological inhibitor (SKI-II) and a genetic knockout mouse model (SK1KO), we demonstrated the key role of the SK1/S1P pathway in mediating the protective effects of volatile anesthetics in the liver and small intestine after bilateral nephrectomy. Using immunohistochemistry, we determined that isoflurane exposure upregulated SK1 in small intestinal crypts. Taken together, these data show that isoflurane activates SK1/S1P signaling in small intestinal crypts to protect the liver and intestine after bilateral nephrectomy.

S1P has multiple roles in cellular signaling and in particular, the “sphingolipid rheostat” balances the prosurvival effects of S1P against the apoptotic effects of sphingosine and ceramide (38). S1P protected intestinal cells from apoptosis via Akt activation (16) and reduced IR injury in the lung (43) and kidney (35). S1P binds to five known G protein-coupled receptors (GPCRs; S1P1-5) (53) and FTY720, a sphingosine analog that is phosphorylated in vivo, activates the S1P1-receptor to produce lymphopenia by reducing lymphocyte egress from lymph nodes. FTY720 is protective in models of liver ischemia (26), bowel ischemia (55), and renal ischemia (13).
SK catalyzes the conversion of sphingosine to S1P, regulating the sphingolipid rheostat, and is stimulated by agents such as agonists of growth factor receptors (e.g., PDGF, VEGF, NGF, and EGF), TGF-β, and TNF-α (17). There are two known isoforms, SK1, which promotes cell growth, and SK2, which inhibits cell growth and promotes apoptosis (14). SK1 mediates ischemic postconditioning in mouse hearts (23) and protects against lipopolysaccharide-induced lung injury via downregulation of JNK (8). Mice deficient in the SK1 enzyme had poor recovery from anaphylaxis and delayed histamine clearance, while mice deficient in the SK2 enzyme had rapid recovery from anaphylaxis (44). In contrast, certain models of inflammation such as Crohn’s disease show a deleterious effect of SK as mice treated with an SK inhibitor (39) or mice deficient in the SK1 enzyme (51) had reduced inflammation and colon damage.

In our model, mice were protected from AKI-induced liver and intestinal injury with exposure to isoflurane after bilateral nephrectomy (i.e., postconditioning). Clinically, volatile anesthetics can be administered outside of the operating room (in the intensive care unit) (52), and this may prove to have therapeutic benefits in patients identified as having suffered from AKI. Anesthetics have multiple physiological effects, including alterations in hemodynamics and regional disturbances in blood flow. We have previously shown that volatile anesthetics did not significantly alter systemic blood pressure, renal blood flow, or core body temperature compared with pentobarbital sodium (30). Metabolites of volatile anesthetics, such as inorganic fluoride from methoxyflurane, have direct nephrotoxic effects, but isoflurane has minimal metabolism and has not been shown to have nephrotoxic effects (7). Volatile anesthetics may have both local and systemic effects in reducing the severity of hepatic and intestinal injury following bilateral nephrectomy, so in an in vivo study it is difficult to distinguish between direct cytoprotective effects on hepatocytes and small intestinal crypts and systemic effects such as leukocyte modulation, preservation of endothelial barrier integrity, and reduction of proinflammatory cytokines.

In conclusion, we have shown that isoflurane activates small intestinal crypt SK1/S1P signaling to reduce hepatic injury and small intestinal apoptosis, necrosis, and inflammation after bilateral nephrectomy. Advancements in treating AKI-mediated extrarenal organ dysfunction may be made as the mechanisms of protection are further elucidated.

GRANTS
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES

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